



# ON THE VARIATION OF THE HAEMOLYTIC COMPLEMENT IN EXPERIMENTAL TRYPANOSOMIASIS

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Hartoch and Yakimoff\* observed a diminution in the amount of haemolytic complement in the blood of guinea-pigs and rats infected with various strains of trypanosomes shortly before the death of the animals.

With a view to ascertaining whether this diminution of the haemolytic complement bore any definite relation to the number of trypanosomes present in the peripheral blood of the infected animal, or whether it was simply a terminal event of the disease, the following series of experiments was undertaken.†

For the purpose of preparing a haemolytic system about 50 c.c. of goat's red corpuscles were injected into the peritoneal cavity of a rabbit, all traces of serum having been previously removed from the red cells by washing three or four times with 0.9 per cent. NaCl solution. These injections were repeated once or twice at intervals of seven days. Usually at the end of two or three weeks the rabbit's serum was strongly haemolytic for goat's red cells. The immune rabbit-serum was then inactivated by heating to 56°-58° C. for half an hour.

In order to standardise the haemolytic system, amounts varying

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\* Wien klin. Woch., No. 40, 1908.

† The animal experiments recorded in this paper were kindly performed for me by Dr. Anton Breinl.

from 0.0005 c.c.-0.005 c.c. of the immune serum were added to 1 c.c. of a 5 per cent suspension of well-washed goat's red cells. Afterwards, 1 c.c. of a 1 in 10 dilution of normal guinea-pig serum was added, as complement, to each of these mixtures of amboceptor and red cells. The contents of each of the tubes was then made up to 3 c.c. by the addition of 0.9 per cent. NaCl solution. It was found that 0.001 c.c. of the immune serum was the smallest amount necessary to haemolyse 1 c.c. of a 5 per cent. suspension of goat's red cells in from 10-15 minutes at 37° C.

A haemolytic system consisting of the double amount of the immune serum 0.002 c.c. and 1 c.c. of a 5 per cent. suspension of goat's red cells was employed in the following experiments.

The serum of infected guinea-pigs was used throughout these experiments. The amount of complement present in the blood, withdrawn from one of the veins in the ear, was estimated before injection, and the guinea-pigs were then inoculated with different strains of trypanosomes, e.g., *T. gambiense*, *T. equiperdum*, *T. brucei* or *T. evansi*. Subsequently, examinations of the amount of haemolytic complement in their serum were made at intervals of two or three days during the course of the infection. An observation of the number of trypanosomes in the peripheral blood was made daily.

In Tables I and II the results obtained with the serum of guinea-pigs infected with *T. brucei* and *T. evansi*, respectively, are recorded.

Similar results were obtained with guinea-pigs 13 and 14 infected with *T. gambiense*, and with guinea-pigs 15 and 16 infected with *T. equiperdum*. The amount of haemolytic complement present in the serum of these animals was examined frequently during the period—between three and four and a half months—which they lived after infection. At no time was any diminution of the complement observed until a few hours before the animal's death, when a partial disappearance of complement was noticed in animals 13 and 15. There was no appreciable loss of complement observed in guinea-pigs 14 and 16 in the serum removed about twelve hours before their death. At no time did their blood exhibit more than one or two trypanosomes to a field, and frequently none were seen after a careful search.

TABLE I—Guinea-pigs infected with *T. brucei*.

| No. of experiment | Day                       | No. of parasites present in blood                  | Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution. |                                 |                                       |                                   |                                       |                                |
|-------------------|---------------------------|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------------|-----------------------------------|---------------------------------------|--------------------------------|
|                   |                           |                                                    | 0.5 c.c.                                                                                                                                                                                                                                                    | 0.1 c.c.                        | 0.075 c.c.                            | 0.05 c.c.                         | 0.025 c.c.                            | 0.01 c.c.                      |
| 1                 | 5 days before inoculation | Inoculated intraperitoneally with <i>T. brucei</i> |                                                                                                                                                                                                                                                             | Complete haemolysis in 15 mins. | Complete haemolysis in 15 mins.       | Complete haemolysis in 20 mins.   | Almost complete haemolysis in 3 hours | Trace of haemolysis in 3 hours |
|                   | 1st day after inoculation |                                                    |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                                | " 20 "                            | " 3 "                                 | " 3 "                          |
|                   | 2nd "                     | 5 parasites to a field                             |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                                | " 20 "                            | " 3 "                                 | " 3 "                          |
|                   | 3rd "                     |                                                    |                                                                                                                                                                                                                                                             |                                 |                                       |                                   |                                       |                                |
|                   | 4th "                     | 10 "                                               |                                                                                                                                                                                                                                                             |                                 |                                       |                                   |                                       |                                |
|                   | 5th "                     | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                       |                                   |                                       |                                |
|                   | 6th "                     | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                       |                                   |                                       |                                |
|                   | 7th "                     | 50 Parasites numerous                              |                                                                                                                                                                                                                                                             | " 30 "                          | Almost complete haemolysis in 3 hours | Very marked haemolysis in 3 hours | Trace of haemolysis in 3 hours        | No haemolysis in 3 hours       |
|                   | 8th "                     |                                                    |                                                                                                                                                                                                                                                             |                                 |                                       |                                   |                                       |                                |
|                   | 9th "                     | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                       |                                   |                                       |                                |
|                   | 10th "                    | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                       |                                   |                                       |                                |
|                   | 11th "                    | 40 to field                                        | Complete haemolysis in 10 mins.                                                                                                                                                                                                                             | " 30 "                          | " 3 "                                 | " 3 "                             | " 3 "                                 | " 3 "                          |
|                   | 12th "                    | Numerous*                                          | No haemolysis in 3 hours                                                                                                                                                                                                                                    | No haemolysis in 3 hours        | No haemolysis in 3 hours              | No haemolysis in 3 hours          | No haemolysis in 3 hours              | No haemolysis in 3 hours       |
|                   | 13th "                    | Found dead                                         |                                                                                                                                                                                                                                                             |                                 |                                       |                                   |                                       |                                |

\* Animal very ill at the time the serum was removed.



TABLE I—continued.

| No. of experiment | Day                       | No. of parasites present in blood                  | Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution. |                                 |                                 |                                 |                                       |                                |
|-------------------|---------------------------|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------------|--------------------------------|
|                   |                           |                                                    | 0.5 c.c.                                                                                                                                                                                                                                                    | 0.1 c.c.                        | 0.075 c.c.                      | 0.05 c.c.                       | 0.025 c.c.                            | 0.01 c.c.                      |
| 2                 | 2 days before inoculation | Inoculated intraperitoneally with <i>T. brucei</i> |                                                                                                                                                                                                                                                             | Complete haemolysis in 10 mins. | Complete haemolysis in 15 mins. | Complete haemolysis in 20 mins. | Almost complete haemolysis in 3 hours | Trace of haemolysis in 3 hours |
|                   | 1st day after inoculation |                                                    |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
|                   | 2nd                       | 5 to a field                                       |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 3rd                       | 2 "                                                |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 4th                       | 5 "                                                |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 5th                       | 10 "                                               |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 6th                       | 15 "                                               |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 7th                       | 5 "                                                |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 8th                       | 1 to 5 fields                                      |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 9th                       | 1 to 20 fields                                     |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 10th                      | 1 to 5 "                                           |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 11th                      | 1 to a field                                       |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 12th                      | 5 "                                                |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 13th                      | 10 "                                               |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 14th                      | 15 "                                               |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 15th                      | 40 "                                               |                                                                                                                                                                                                                                                             | " 15 "                          | " 15 "                          | " 20 "                          | " 3 "                                 | " 3 "                          |
|                   | 16th                      | Numerous                                           | Complete haemolysis in 10 mins.                                                                                                                                                                                                                             | No haemolysis in 3 hours        | No haemolysis in 3 hours        | No haemolysis in 3 hours        | No haemolysis in 3 hours              | No haemolysis in 3 hours       |
|                   | 17th                      |                                                    | Slight haemolysis in 3 hours                                                                                                                                                                                                                                | No haemolysis in 3 hours        | No haemolysis in 3 hours        | No haemolysis in 3 hours        | No haemolysis in 3 hours              | No haemolysis in 3 hours       |
|                   | 18th                      | Immunisable*                                       |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
|                   | 19th                      | Found dead                                         |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
|                   | 20th                      |                                                    |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |

\* Animal very ill at the time the serum was removed.

TABLE I—continued.

| No. of experiment | Day                       | No. of parasites present in blood                   | Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution. |                                 |                                       |                                 |                                       |                              |                          |
|-------------------|---------------------------|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------------|---------------------------------|---------------------------------------|------------------------------|--------------------------|
|                   |                           |                                                     | 0.5 c.c.                                                                                                                                                                                                                                                      | 0.1 c.c.                        | 0.075 c.c.                            | 0.05 c.c.                       | 0.025 c.c.                            | 0.01 c.c.                    | 0.005 c.c.               |
| 3                 |                           | Inoculated intra-peritoneally with <i>T. brucei</i> |                                                                                                                                                                                                                                                               | Complete haemolysis in 10 mins. | Complete haemolysis in 15 mins.       | Complete haemolysis in 20 mins. | Almost complete haemolysis in 3 hours | Slight haemolysis in 3 hours | No haemolysis in 3 hours |
|                   | 1st day after inoculation |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 2nd "                     |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 3rd "                     |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 4th "                     | 5 to a field                                        |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 5th "                     |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 6th "                     | 30 "                                                |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 7th "                     | 20 "                                                |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 8th "                     | 1 to 5 fields                                       |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 9th "                     |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 10th "                    | 1 to 20 fields                                      |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 11th "                    | 1 to a field                                        |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 12th "                    | 5 "                                                 |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 13th "                    | 10 "                                                |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 14th "                    | 15 "                                                |                                                                                                                                                                                                                                                               | " 10 "                          | " 15 "                                | " 20 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 15th "                    | 40 "                                                |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 16th "                    |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 17th "                    | Innumerable                                         | Complete haemolysis in 20 mins.                                                                                                                                                                                                                               | " 60 "                          | Almost complete haemolysis in 3 hours | Slight haemolysis in 3 hours    | No haemolysis in 3 hours              | No haemolysis in 3 hours     | " 3 "                    |
|                   | 18th "                    |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 19th "                    |                                                     | No haemolysis in 3 hours                                                                                                                                                                                                                                      | No haemolysis in 3 hours        | No haemolysis in 3 hours              | No haemolysis in 3 hours        | " 3 "                                 | " 3 "                        | " 3 "                    |

\* Animal dying when the serum was withdrawn.

TABLE I—continued.

| No. of experiment | Day                       | No. of parasites present in blood                  | Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution. |                                 |                                 |                                 |                                       |                              |                          |
|-------------------|---------------------------|----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------------|------------------------------|--------------------------|
|                   |                           |                                                    | 0.5 c.c.                                                                                                                                                                                                                                                      | 0.1 c.c.                        | 0.075 c.c.                      | 0.05 c.c.                       | 0.025 c.c.                            | 0.01 c.c.                    | 0.005 c.c.               |
| 4                 |                           | Inoculated intraperitoneally with <i>T. brucei</i> |                                                                                                                                                                                                                                                               | Complete haemolysis in 15 mins. | Complete haemolysis in 15 mins. | Complete haemolysis in 30 mins. | Almost complete haemolysis in 3 hours | Slight haemolysis in 3 hours | No haemolysis in 3 hours |
|                   | 1st day after inoculation |                                                    |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 2nd ..                    |                                                    |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 3rd ..                    | 1 to 2 fields                                      |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 4th ..                    | 10 to a field                                      |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 5th ..                    | 20 "                                               |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 6th ..                    | 30 "                                               |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 7th ..                    | 20 "                                               |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 8th ..                    | 20 "                                               |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 9th ..                    |                                                    |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 10th ..                   | Found dead                                         |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |

| No. of experiment | Day                       | No. of parasites present in blood                   | Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution. |                                 |                                       |                                       |                                       |                              |                          |
|-------------------|---------------------------|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|------------------------------|--------------------------|
|                   |                           |                                                     | 0.5 c.c.                                                                                                                                                                                                                                                      | 0.1 c.c.                        | 0.075 c.c.                            | 0.05 c.c.                             | 0.025 c.c.                            | 0.01 c.c.                    | 0.005 c.c.               |
| 5                 |                           | Inoculated intra-peritoneally with <i>T. brucei</i> |                                                                                                                                                                                                                                                               | Complete haemolysis in 15 mins. | Complete haemolysis in 15 mins.       | Complete haemolysis in 30 mins.       | Almost complete haemolysis in 3 hours | Slight haemolysis in 3 hours | No haemolysis in 3 hours |
|                   | 1st day after inoculation | 4 to film                                           |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 2nd "                     | 1 to field                                          |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 3rd "                     | 10 "                                                |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 20 "                                | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 4th "                     | 40 "                                                |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 5th "                     |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 6th "                     |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 7th "                     | Numerous                                            |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 8th "                     | "                                                   |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 30 "                                | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 9th "                     | "                                                   |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 10th "                    | "                                                   |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 11th "                    | "                                                   |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 30 "                                | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 12th "                    | "                                                   |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 13th "                    |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 14th "                    | 1 to a field                                        |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 15th "                    | 2 "                                                 |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 16th "                    | 5 "                                                 |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 17th "                    | 10 "                                                |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 30 "                                | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 18th "                    | 15 "                                                |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 19th "                    |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 20th "                    | 40 "                                                |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | Almost complete haemolysis in 3 hours | Partial haemolysis in 3 hours         | No haemolysis in 3 hours     | " 3 "                    |
|                   | 21st "                    | Found dead                                          |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
| 6                 |                           | Inoculated intra-peritoneally with <i>T. brucei</i> |                                                                                                                                                                                                                                                               | Complete haemolysis in 15 mins. | Complete haemolysis in 15 mins.       | Complete haemolysis in 30 mins.       | Marked haemolysis in 3 hours          | No haemolysis in 3 hours     | No haemolysis in 3 hours |
|                   | 1st day after inoculation |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 2nd "                     |                                                     |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 3rd "                     | 1 to a field                                        |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 4th "                     | 10 "                                                |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 5th "                     | 50 "                                                |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 30 "                                | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 6th "                     | 50 "                                                |                                                                                                                                                                                                                                                               |                                 |                                       |                                       |                                       |                              |                          |
|                   | 7th "                     | Numerous*                                           | Complete haemolysis in 10 mins.                                                                                                                                                                                                                               | " 15 "                          | Almost complete haemolysis in 3 hours | Slight haemolysis in 3 hours          | No haemolysis in 3 hours              | " 3 "                        | " 3 "                    |

\* Animal dying when the serum was withdrawn.



TABLE 1 continued.

| No. of experiment | Day                       | No. of parasites present in blood                  | Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution. |                                 |                                       |                                 |                                       |                              |                          |
|-------------------|---------------------------|----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------------|---------------------------------|---------------------------------------|------------------------------|--------------------------|
|                   |                           |                                                    | 0.5 c.c.                                                                                                                                                                                                                                                      | 0.1 c.c.                        | 0.075 c.c.                            | 0.05 c.c.                       | 0.025 c.c.                            | 0.01 c.c.                    | 0.005 c.c.               |
| 7                 |                           | Inoculated intraperitoneally with <i>T. brucei</i> |                                                                                                                                                                                                                                                               | Complete haemolysis in 15 mins. | Complete haemolysis in 15 mins.       | Complete haemolysis in 20 mins. | Almost complete haemolysis in 3 hours | Slight haemolysis in 3 hours | No haemolysis in 3 hours |
|                   | 1st day after inoculation |                                                    |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 2nd "                     |                                                    |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 3rd "                     |                                                    |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 4th "                     | 10 to a field                                      |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 5th "                     |                                                    |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 6th "                     | 5 "                                                |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 7th "                     | 1 to 5 fields                                      |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 8th "                     | 1 to 10 "                                          |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 9th "                     | 1 to 5 "                                           |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 10th "                    |                                                    |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 11th "                    | 1 to a field                                       |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 12th "                    | 10 "                                               |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 13th "                    | 10 "                                               |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 14th "                    | 10 "                                               |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 15th "                    | 10 "                                               |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 16th "                    | 15 "                                               |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 17th "                    | 40 "                                               |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 18th "                    | Numerous                                           | Complete haemolysis in 10 mins.                                                                                                                                                                                                                               | " 15 "                          | " 15 "                                | " 20 "                          | " 3 "                                 | No haemolysis in 3 hours     | " 3 "                    |
|                   | 19th "                    | 15 to a field                                      |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 20th "                    | 50 "                                               |                                                                                                                                                                                                                                                               |                                 |                                       |                                 |                                       |                              |                          |
|                   | 21st "                    | Numerous                                           |                                                                                                                                                                                                                                                               | " 20 "                          | " 20 "                                | " 30 "                          | " 3 "                                 | Slight haemolysis in 3 hours | " 3 "                    |
|                   | 22nd "                    | "                                                  | " 10 "                                                                                                                                                                                                                                                        | " 15 "                          | " 15 "                                | " 20 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 23rd "                    | " *                                                | " 10 "                                                                                                                                                                                                                                                        | " 60 "                          | Almost complete haemolysis in 3 hours | Slight haemolysis in 3 hours    | No haemolysis in 3 hours              | No haemolysis in 3 hours     | " 3 "                    |

\* Animal dying when the serum was withdrawn.

TABLE I—continued.

| No. of experiment | Day                       | No. of parasites present in blood                   | Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution. |          |            |           |            |            |
|-------------------|---------------------------|-----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|------------|-----------|------------|------------|
|                   |                           |                                                     | 0.5 c.c.                                                                                                                                                                                                                                                    | 0.1 c.c. | 0.075 c.c. | 0.05 c.c. | 0.025 c.c. | 0.01 c.c.  |
| 8                 |                           |                                                     |                                                                                                                                                                                                                                                             |          |            |           |            | 0.005 c.c. |
|                   |                           | Inoculated intra-peritoneally with <i>T. brucei</i> |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 1st day after inoculation | 1 to 20 fields                                      |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 2nd "                     | 1 to 10 "                                           |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 3rd "                     | 5 to field                                          |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 4th "                     | 2 "                                                 |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 5th "                     | 15 "                                                |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 6th "                     | "                                                   | 15 "                                                                                                                                                                                                                                                        |          | 15 "       | 30 "      | 3 "        | 3 "        |
|                   | 7th "                     | 5 "                                                 |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 8th "                     | 1 "                                                 |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 9th "                     | 5 "                                                 | 15 "                                                                                                                                                                                                                                                        |          | 15 "       | 30 "      | 3 "        | 3 "        |
|                   | 10th "                    | 15 "                                                |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 11th "                    | Numerous                                            | 15 "                                                                                                                                                                                                                                                        |          | 15 "       | 30 "      | 3 "        | 3 "        |
|                   |                           | Complete haemolysis in 10 mins.                     |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 12th "                    | " 10 "                                              | 15 "                                                                                                                                                                                                                                                        |          | 15 "       | 30 "      | 3 "        | 3 "        |
|                   | 13th "                    | "                                                   |                                                                                                                                                                                                                                                             |          |            |           |            |            |
|                   | 14th "                    | Found dead                                          |                                                                                                                                                                                                                                                             |          |            |           |            |            |

Almost complete haemolysis in 3 hours

Slight haemolysis in 3 hours

No haemolysis in 3 hours

Complete haemolysis in 30 mins.

Complete haemolysis in 15 mins.

Complete haemolysis in 15 mins.

Complete haemolysis in 10 mins.

TABLE I—continued

| No. of experiment | Day                       | No. of parasites present in blood                  | Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5% suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9% NaCl solution. |                                 |                                 |                                 |                                       |                                |
|-------------------|---------------------------|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------------|--------------------------------|
|                   |                           |                                                    | 0.5 c.c.                                                                                                                                                                                                                                                    | 0.1 c.c.                        | 0.075 c.c.                      | 0.05 c.c.                       | 0.025 c.c.                            | 0.005 c.c.                     |
|                   |                           |                                                    |                                                                                                                                                                                                                                                             | Complete haemolysis in 15 mins. | Complete haemolysis in 15 mins. | Complete haemolysis in 30 mins. | Almost complete haemolysis in 3 hours | Trace of haemolysis in 3 hours |
|                   |                           |                                                    |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       | No haemolysis in 3 hours       |
| 1                 | 1st day after inoculation | Inoculated intraperitoneally with <i>T. brucei</i> |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 2nd               | "                         | 1 to 40 fields                                     |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 3rd               | "                         | 1 to 1 film                                        |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 4th               | "                         | 1 to 5 fields                                      | 15 "                                                                                                                                                                                                                                                        | "                               | 15 "                            | 30 "                            | 3 "                                   | 3 "                            |
| 5th               | "                         | 1 to 15 fields                                     |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 6th               | "                         | 1 to 30 fields                                     |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 7th               | "                         | 1 to 1 film                                        |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 8th               | "                         | 1 to 5 fields                                      | 15 "                                                                                                                                                                                                                                                        | "                               | 15 "                            | 30 "                            | 3 "                                   | 3 "                            |
| 9th               | "                         | 5-10 to field                                      |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 10th              | "                         | 1 to 2 fields                                      |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 11th              | "                         | 1 to 1 film                                        |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 12th              | "                         | 1 to 1 film                                        |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 13th              | "                         | 1 to 1 film                                        | 15 "                                                                                                                                                                                                                                                        | "                               | 15 "                            | 30 "                            | 3 "                                   | 3 "                            |
| 14th              | "                         | 1 to 1 film                                        |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 15th              | "                         | 1 to film                                          |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 16th              | "                         | 1 to film                                          |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 17th              | "                         | Negative                                           |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 18th              | "                         | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 19th              | "                         | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 20th              | "                         | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 21st              | "                         | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 22nd              | "                         | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 23rd              | "                         | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 24th              | "                         | 1 to film                                          |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 25th              | "                         | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 26th              | "                         | "                                                  |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 27th              | "                         | 1 to 5 fields                                      |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 28th              | "                         | 1 to 20 "                                          | 15 "                                                                                                                                                                                                                                                        | "                               | 15 "                            | 30 "                            | 3 "                                   | 3 "                            |
| 29th              | "                         | 1 to 5 "                                           |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 30th              | "                         | 1 to 10 "                                          |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 31st              | "                         | 1 to 5 "                                           |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 32nd              | "                         | 10 to field                                        |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 33rd              | "                         | 15 "                                               |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 34th              | "                         | 15 "                                               | 15 "                                                                                                                                                                                                                                                        | "                               | 15 "                            | 30 "                            | 3 "                                   | 3 "                            |
| 35th              | "                         | 50 "                                               |                                                                                                                                                                                                                                                             |                                 |                                 |                                 |                                       |                                |
| 36th              | "                         | Numerous                                           | 15 "                                                                                                                                                                                                                                                        | "                               | 15 "                            | 30 "                            | 3 "                                   | 3 "                            |
| 37th              | "                         | "                                                  | No haemolysis in 3 hours                                                                                                                                                                                                                                    | No haemolysis in 5 hours        | No haemolysis in 3 hours        | No haemolysis in 3 hours        | No haemolysis in 3 hours              | No haemolysis in 3 hours       |

\* Animal moribund at the time the serum was withdrawn.

TABLE II.—Guinea-pigs infected with *T. evansi*.

| No. of experiment | Day                       | No. of parasites present in blood                   | Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution. |                                 |                                 |                                 |                                       |                              |                          |  |
|-------------------|---------------------------|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------------|------------------------------|--------------------------|--|
|                   |                           |                                                     | 0.5 c.c.                                                                                                                                                                                                                                                      | 0.1 c.c.                        | 0.075 c.c.                      | 0.05 c.c.                       | 0.025 c.c.                            | 0.01 c.c.                    | 0.005 c.c.               |  |
| 10                |                           | Inoculated intra-peritoneally with <i>T. evansi</i> |                                                                                                                                                                                                                                                               | Complete haemolysis in 15 mins. | Complete haemolysis in 15 mins. | Complete haemolysis in 30 mins. | Almost complete haemolysis in 3 hours | Slight haemolysis in 3 hours | No haemolysis in 3 hours |  |
|                   | 1st day after inoculation | Negative                                            |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |  |
|                   | 2nd "                     | 1 to field                                          |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |  |
|                   | 3rd "                     | 5 "                                                 |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |  |
|                   | 4th "                     | 10 "                                                |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |  |
|                   | 5th "                     | Negative                                            |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |  |
|                   | 6th "                     | 2 to film                                           |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |  |
|                   | 7th "                     | 1 to 2 fields                                       |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |  |
|                   | 8th "                     | 20 to field                                         |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |  |
|                   | 9th "                     | Found dead                                          |                                                                                                                                                                                                                                                               | " 15 "                          | " 15 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |  |
| 11                |                           | Inoculated intra-peritoneally with <i>T. evansi</i> | Complete haemolysis in 10 mins.                                                                                                                                                                                                                               | Complete haemolysis in 15 mins. | Complete haemolysis in 20 mins. | Complete haemolysis in 30 mins. | Marked haemolysis in 3 hours          | No haemolysis in 3 hours     | No haemolysis in 3 hours |  |
|                   | 1st day after inoculation |                                                     |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |  |
|                   | 2nd "                     |                                                     |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |  |
|                   | 3rd "                     | 5 to field                                          |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |  |
|                   | 4th "                     | 15 "                                                |                                                                                                                                                                                                                                                               | " 15 "                          | " 20 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |  |
|                   | 5th "                     | 15 "                                                |                                                                                                                                                                                                                                                               | " 15 "                          | " 20 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |  |
|                   | 6th "                     | 10 "                                                | " 10 "                                                                                                                                                                                                                                                        | " 15 "                          | " 15 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |  |
|                   | 7th "                     | Found dead                                          | " 10 "                                                                                                                                                                                                                                                        | " 15 "                          | " 15 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |  |



TABLE II—continued.

| No. of experiment | Day                       | No. of parasites present in blood                  | Amount of guinea-pig serum (complement) added to the standardised haemolytic system, consisting of 0.002 c.c. amboceptor and 1 c.c. of a 5 % suspension of red cells. In each case the contents of the tubes were made up to 3 c.c. with 0.9 % NaCl solution. |                                 |                                 |                                 |                                       |                              |                          |
|-------------------|---------------------------|----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------------|------------------------------|--------------------------|
|                   |                           |                                                    | 0.5 c.c.                                                                                                                                                                                                                                                      | 0.1 c.c.                        | 0.075 c.c.                      | 0.05 c.c.                       | 0.025 c.c.                            | 0.01 c.c.                    | 0.005 c.c.               |
| 12                |                           | Inoculated intraperitoneally with <i>T. evansi</i> | Complete haemolysis in 10 mins.                                                                                                                                                                                                                               | Complete haemolysis in 15 mins. | Complete haemolysis in 20 mins. | Complete haemolysis in 30 mins. | Almost complete haemolysis in 3 hours | Slight haemolysis in 3 hours | No haemolysis in 3 hours |
|                   | 1st day after inoculation | Negative                                           |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 2nd "                     | "                                                  |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 3rd "                     | 2 to film                                          |                                                                                                                                                                                                                                                               | " 15 "                          | " 20 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 4th "                     | "                                                  |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 5th "                     | 1 to field                                         |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 6th "                     | "                                                  |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 7th "                     | 15 "                                               |                                                                                                                                                                                                                                                               | " 15 "                          | " 20 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 8th "                     | 15 "                                               |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 9th "                     | 15 "                                               |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 10th "                    | 20 "                                               |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 11th "                    | 30 "                                               |                                                                                                                                                                                                                                                               | " 15 "                          | " 20 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 12th "                    | 2 "                                                |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 13th "                    | 1 to 5 fields                                      |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 14th "                    | 1 to 10 fields                                     |                                                                                                                                                                                                                                                               | " 15 "                          | " 20 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 15th "                    | 5 to field                                         |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 16th "                    | 1 "                                                |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 17th "                    | 3 "                                                |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 18th "                    | 4 to film                                          |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 19th "                    | 5 "                                                |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 20th "                    | Negative                                           |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 21st "                    | "                                                  |                                                                                                                                                                                                                                                               | " 15 "                          | " 20 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 22nd "                    | "                                                  |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 23rd "                    | 2 to film                                          |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 24th "                    | 2 "                                                |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 25th "                    | Negative                                           |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 26th "                    | 1 to 5 fields                                      |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 27th "                    | "                                                  |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 28th "                    | 10 to field                                        | " 10 "                                                                                                                                                                                                                                                        | " 15 "                          | " 20 "                          | " 30 "                          | " 3 "                                 | " 3 "                        | " 3 "                    |
|                   | 29th "                    | 60 "                                               |                                                                                                                                                                                                                                                               |                                 |                                 |                                 |                                       |                              |                          |
|                   | 30th "                    | Numerous *                                         | No haemolysis in 3 hours                                                                                                                                                                                                                                      | No haemolysis in 3 hours        | No haemolysis in 3 hours        | No haemolysis in 3 hours        | No haemolysis in 3 hours              | No haemolysis in 3 hours     | No haemolysis in 3 hours |

\* Animal dying at the time the serum was withdrawn.

In none of the foregoing experiments was any decrease in the amount of haemolytic complement observed until shortly before the death of the animal, with the single exception of Experiment 1. The serum of this guinea-pig, which was infected with *T. brucei*, showed a slight decrease in complement on the 8th and 11th day of the disease. The blood at the time contained numerous trypanosomes.

In Experiments 2, 5, 7 and 8 no appreciable decrease of the haemolytic complement occurred during the course of the disease, even when the parasites were swarming in the peripheral blood (1 to every 2 or 3 red cells). Many estimations of the complement were made in the different experiments, when 20-50 parasites were present to the microscopic field (Zeiss objective DD; eyepiece No. 4). In no case was any diminution noticed. It is evident, therefore, that the presence of numerous trypanosomes in the peripheral blood in an early stage of the disease does not necessarily result in a diminution of the haemolytic complement.

On the other hand in a late stage of the infection shortly before the death of the animal, a considerable decrease in the amount of complement was observed in most of the experiments. On five occasions, Experiments 1, 2, 3, 9 and 12, the blood of animals infected with *T. brucei* and *T. evansi*, respectively, contained practically no haemolytic complement a few hours before death. In other cases, e.g., Experiments 6, 7, 13 and 15, only a partial diminution could be observed in the serum removed just before the death of the animal, whilst in still other cases, 5, 10, 11, 14 and 16, no decrease of the haemolytic complement was found in the serum withdrawn about twelve hours before death.

From these observations one must conclude, therefore, that the diminution of the complement in the serum of animals infected with trypanosomiasis is simply a terminal event, and does not occur during the earlier stages of the disease, even when the peripheral blood is swarming with parasites.

The following experiments were performed with a view to ascertaining whether the serum of infected guinea-pigs withdrawn a few hours before their death contained any body which had the property of inhibiting to any extent the activating power of the complement of normal serum.

EXPERIMENT. — The serum of guinea-pig 3 was obtained shortly before the death of the animal. It had not the slightest activating action even when added undiluted to the standardised haemolytic system of goat's red cells and amboceptor. A portion of this serum was added in varying proportions to the serum of a normal guinea-pig. The resulting mixtures were then kept at 37° C. for 20 minutes before being added to the haemolytic system. A second portion of the serum of guinea-pig 3 was first heated to 56°-58° C. for half an hour in order to destroy any complement it might contain, and then, as above, mixed in different proportions with normal guinea-pig serum.

The following Table gives the result of this experiment :

A

|   | 5 %<br>suspension<br>of goat's red<br>cells | Amboceptor | COMPLEMENT                    |                        | Haemolysis                      |
|---|---------------------------------------------|------------|-------------------------------|------------------------|---------------------------------|
|   |                                             |            | Normal<br>guinea-pig<br>serum | 0.9 % NaCl<br>solution |                                 |
| 1 | 1.0 c.c.                                    | 0.002 c.c. | 0.1 c.c.                      | 0.1 c.c.               | Complete haemolysis in 15 mins. |
| 2 | "                                           | "          | 0.05 c.c.                     | 0.15 c.c.              | Complete haemolysis in 30 mins. |
| 3 | "                                           | "          | 0.025 c.c.                    | 0.175 c.c.             | Partial haemolysis in 3 hours   |
| 4 | "                                           | "          | 0.01 c.c.                     | 0.19 c.c.              | Trace of haemolysis in 3 hours  |

B

|   | 5 %<br>suspension<br>of goat's red<br>cells | Amboceptor | COMPLEMENT                    |                          | Haemolysis                               |
|---|---------------------------------------------|------------|-------------------------------|--------------------------|------------------------------------------|
|   |                                             |            | Normal<br>guinea-pig<br>serum | Serum<br>guinea-pig<br>3 |                                          |
| 1 | 1.0 c.c.                                    | 0.002 c.c. | 0.1 c.c.                      | 0.1 c.c.                 | Complete haemolysis in 15 mins.          |
| 2 | "                                           | "          | 0.05 c.c.                     | 0.15 c.c.                | Complete haemolysis in 30 mins.          |
| 3 | "                                           | "          | 0.025 c.c.                    | 0.175 c.c.               | Partially complete haemolysis in 3 hours |
| 4 | "                                           | "          | 0.01 c.c.                     | 0.19 c.c.                | Slight haemolysis in 3 hours             |

C

|   | 5 %<br>suspension<br>of goat's red<br>cells | Amboceptor | COMPLEMENT                    |                                                                      | Haemolysis                      |
|---|---------------------------------------------|------------|-------------------------------|----------------------------------------------------------------------|---------------------------------|
|   |                                             |            | Normal<br>guinea-pig<br>serum | Serum of<br>guinea-pig<br>3<br>(heated<br>56-58° C. for<br>20 mins.) |                                 |
| 1 | 1.0 c.c.                                    | 0.002 c.c. | 0.1 c.c.                      | 0.1 c.c.                                                             | Complete haemolysis in 15 mins. |
| 2 | "                                           | "          | 0.05 c.c.                     | 0.15 c.c.                                                            | Complete haemolysis in 30 mins. |
| 3 | "                                           | "          | 0.025 c.c.                    | 0.175 c.c.                                                           | Partial haemolysis in 3 hours   |
| 4 | "                                           | "          | 0.01 c.c.                     | 0.19 c.c.                                                            | Trace of haemolysis in 3 hours  |